Case Report Rapport de cas

Bovine leukemia virus infection in a juvenile alpaca with multicentric lymphoma

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Abstract — A 13-month-old alpaca *(Vicugna pacos)* was presented for mandibular masses and weight loss. Histopathology of biopsy tissue was consistent with lymphoma. The alpaca was euthanized and necropsy revealed lymphoma masses in multiple organs. Immunohistochemistry for T- and B-cell typing was inconclusive. Serology and in-situ polymerase chain reaction hybridization were positive for bovine leukemia virus.

Résumé — Infection par le virus de la leucémie bovine chez un alpaga juvénile atteint d'un lymphome multicentrique. Un alpaga *(Vicugna pacos)* âgé de 13 mois a été présenté pour des masses mandibulaires et une perte de poids. L'histopathologie des tissus de biopsie était conforme au lymphome. L'alpaga a été euthanasié et la nécropsie a révélé des masses de lymphome dans de multiples organes. L'immunohistochimie pour le typage des cellules T et B n'a pas été concluant. La sérologie et l'hybridation in situ par réaction d,amplification en chaîne par la polymérase étaient positives pour le virus de la leucémie bovine.

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Lymphoma is the most commonly reported neoplasm of new world camelids (NWC) (1,2). Bovine leukemia virus (BLV), an oncogenic virus belonging to the Retroviridae family, is associated with lymphoma in cattle (3). This association led others to investigate cases of lymphoma in NWC for the presence of BLV, but none was detected using electron microscopy (1,4). This is the first report of lymphoma in an alpaca which tested positive for BLV by serology and in-situ-polymerase chain reaction (IS-PCR).

Case description

A 13-month-old intact male Huacaya alpaca *(Vicugna pacos)* was presented for evaluation of 2 mandibular masses and weight loss of 2 weeks duration. Prior to presentation, the alpaca had been treated for a suspected tooth root abscess with enrofloxacin [3 mg/kg body weight (BW), subcutaneously] and ketoprofen (1.1 mg/kg BW, intramuscularly). The animal originated from a farm in Virginia, USA with 250 alpacas and was kept in a field with 2 other alpacas.

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The alpaca was underweight (body condition score 2/5, body weight 49.5 kg, average age-matched alpaca weight 63 kg). It had a marginally subnormal rectal temperature of 37.2°C, normal heart rate of 64 beats/min, and an elevated respiratory rate of 36 breaths/min with no increased respiratory effort. The mandibular masses consisted of a firm, ovoid, subcutaneous mass (2 cm × 1 cm) on the caudo-lateral aspect of the right mandible and a raised, ulcerated, gingival mass (2 cm × 1.5 cm) closely associated with, and causing displacement of the left incisors. The right mandibular lymph node was also enlarged (5 cm × 5 cm). The alpaca urinated a full stream of red urine followed by small blood clots. Abdominal palpation revealed enlarged kidneys.

Diagnostic tests included a complete blood (cell) count (CBC), serum biochemistry panel, ultrasound and radiographic examination of the mandible, and biopsies of the mandibular masses for histopathological examination.

The CBC and serum chemistry demonstrated marked azotemia [creatinine 158.4 μmol/L, reference interval (RI): 114.9–256.4 μmol/L], urea nitrogen [72.5 μmol/L (RI: 1.8–10.7 μmol/L)], mild hypoproteinemia [total protein 50 g/L (RI: 51–78 g/L)], and hypoalbuminemia [25 g/L (RI: 35–44 g/L)], with mild leukocytosis and anemia. Urinalysis confirmed hematuria, with intact red blood cells present on sediment evaluation, a specific gravity of 1.017, marked proteinuria, trace glucosuria and 3 to 10 white cells per high power field.

Ultrasound examination of the mandibular masses revealed well-defined areas with homogeneous hyper-echogenicity. Radiographically, the clinically apparent displacement of the left incisors was associated with normal bony structures. Abdominal ultrasonography and radiography confirmed enlarged kidneys, with multiple hyper-echoic nodular lesions present diffusely

throughout the parenchyma, and enlarged mesenteric lymph nodes. The results of thoracic ultrasonography and radiology were unremarkable.

Pending the results of cytology and histopathology, the alpaca was treated with ceftiofur sodium (Naxcel; Pharmacia and Upjohn Company LLC, New York, New York, USA), 2.2 mg/kg BW, IV, q12h, omeprazole (Omeprazole for IV administration, Premier Pharmacy, Weeki Wachee, Florida), 1 mg/kg BW, IV, q24h and balanced isotonic intravenous fluid therapy (Lactated Ringer's solution; Baxter Healthcare USP, Deerfield, Illinois, USA), 4 mL/kg BW per hour.

On day 2, the alpaca was inappetant and lethargic. Repeated laboratory evaluation demonstrated severe azotemia and persistent hematuria. By this time, cytology and histopathology of the mandibular masses had demonstrated lesions consistent with small cell lymphoma. The alpaca was humanely euthanized because of the poor prognosis associated with lymphoma in NWC; the median survival time following recognition of the disease has been reported as 1 month (range 1 wk to 3 mo) (1), with a shorter clinical course in young versus old animals (5).

Necropsy revealed variably sized, mostly homogeneously pale, masses at several sites: the gingiva of the left rostral mandible (2 cm × 1 cm); the right caudo-lateral aspect of the mandible (2 cm × 1.5 cm); on the surface of the liver; within enlarged mesenteric lymph nodes; multiple nodules scattered throughout both kidneys; surrounding the left kidney and adherent to the spleen; within the wall of the caudal vena cava; and attached to the omentum. Both kidneys were enlarged; the left was 14 × 25 × 19 cm with a weight of 2 kg, and the right was 18 × 11 × 12 cm with a weight of 1 kg.

Histopathology of all masses showed sheets of monomorphic round cells with distinct cell borders, scant basophilic cytoplasm, and large round stippled nuclei. The neoplastic cells had moderate anisokaryosis and 2 to 5 mitotic figures per 400× field. There was scattered single cell necrosis throughout the masses. Neoplastic cells infiltrated the pancreas, kidney, liver, omentum, and caudal vena cava. The diagnosis was disseminated small cell lymphoma.

Immunohistochemistry of the mandibular and liver masses was performed using antibodies against B (CD79α)- and T (CD3)-cell markers. Both CD79α and CD3 antibodies stained 5% of the round cells in the mandibular samples with focal areas of staining amounting to about 10% of the fields. The lymphoid cells from the liver had positive staining for CD79α in 10% to 15% of the cells; about 1% of the cells were CD3 positive. The conclusion was that the masses represented lymphoma of undetermined primary cell type, as most cells were not labelled with the markers used.

Serum collected antemortem and submitted to the Virginia Diagnostic Laboratory (VDL) was tested for antibody to BLV using an enzyme-linked immunosorbent assay (ELISA), (Veterinary Medical Research and Development, Pullman, Washington, USA) and agar gel immunodiffusion test (AGIDT), (Veterinary Diagnostic Technology Inc, Colorado, USA). Both assays showed positive results.

Subsequently, neoplastic cells from the alpaca were tested for BLV using IS-PCR hydridization, a modification of an IS-PCR method previously used to successfully detect BLV in bovine tissues (modifications available from authors by request) (6). Specificity of the PCR assay for BLV was determined by previously showing lack of cross-reactivity with other retroviruses (Table 1). The IS-PCR was performed on deparaffinized formalin-fixed tissue sections of omentum, liver, kidney, the right mandibular mass, and pancreas. Primer sequences and PCR conditions were identical to those described previously (6). Positive and negative controls run simultaneously and under identical conditions as the experimental samples gave the expected positive and negative reactions, respectively. Positive controls included a smear of BLV-positive fetal lamb kidney (FLK) cells and a section of known BLV-positive mammary gland tissue from a cow with lymphoma. Negative controls included a smear of FLK and an adjacent serial section of each alpaca tissue reacted with the PCR mixture minus the primers. Specimens of liver and the right mandibular mass contained areas positive for BLV proviral DNA in normal tissue adjacent to the neoplastic lymphoid area. The omentum showed a weakly positive reaction within normal tissue. Bovine leukemia virus was not detected in the pancreas or kidney.

Discussion

Lymphoma is reported to be one of the most common tumors in NWC, although, without confirmatory immunohistochemistry, 25% of cases may have been misdiagnosed as cases of primitive malignant round cell tumors (1,2,7). There is no age or gender predisposition; the age of affected animals in 1 retrospective study ranged from 0 (fetus) to 23 y (7). In the case reported here, the presenting clinical signs of weight loss and palpable masses are consistent with the most common presenting signs in another report on a series of cases (1). The additional observation of hematuria is a clinical feature that has not previously been reported in cases of lymphoma in NWC. Clinical pathology was consistent with other reports, but not necessarily a specific indicator of the tissue type affected (1,5,8). Ultrasonography and radiology were useful imaging modalities that indicated involvement of abdominal tissues, in addition to the mandibular masses that were detected on clinical examination. Previously, radiography has been used in NWC to demonstrate pleural effusion and lung lesions associated with lymphoma (9,10), but ultrasonography has indicated more accurately the location of potentially neoplastic tissues (5). Recent developments in diagnostic imaging techniques include computed tomography, which facilitates an even more precise assessment of tumor location (8). The definitive diagnosis was determined by histopathologic examination of neoplastic masses and the distribution of lesions was consistent with multicentric lymphoma, similar to most reports of lymphoma in NWC (1,4,7–14).

Immunohistochemical analysis has been performed on tissues from NWC with lymphoma to further determine the lymphocyte sub-type; T-cell, B-cell, and mixed tumors have been described mostly by using anti-CD3 for T-cells and anti-CD79α for B-cells (4,7,12–14). Unfortunately, in the alpaca described here, the results of immunohistochemistry did not provide a definitive identification by using these cell markers alone.

Table 1. Lack of cross-reactivity of primers from the bovine leukemia virus *tax* region with other retroviruses in standard solution PCR assays

Retrovirus	Retroviridae genus	Source of virus	Results with *tax* primers
RSV[a] (Rous sarcoma virus)	Alpharetrovirus	XC, a rat cell line transformed with RSV	−
MSV[a] (murine sarcoma virus)	Alpharetrovirus	F81 cat cell line	−
MMTV[a] (mouse mammary tumor virus)	Betaretrovirus	GR mouse cell line	−
MPMV[a] (Mason-Pfizer monkey virus)	Betaretrovirus	CMMT rhesus monkey cell line	−
MuLV (murine leukemia virus)	Gammaretrovirus	JLSV5 mouse cell line	−
FeLV (feline leukemia virus)	Gammaretrovirus	FeLV 3281 cat cell line	−
BLV[a] (bovine leukemia virus)	Deltaretrovirus	FLK sheep cell line	+
		Bat_2Clone_6 cell line	+
		Tb_1Lu (parental line of Bat_2Clone_6 before it was infected with BLV)	−
HTLV-1[a] (human T-cell leukemia virus 1)	Deltaretrovirus	MT2 human lymphocyte cell line	−
HTLV-2[a] (human T-cell leukemia virus 2)	Deltaretrovirus	Clone 19 human lymphocyte cell line	−
STLV (Simian T-cell leukemia virus)	Deltaretrovirus	KIA baboon cell line	−
HIV-1[a] (human immuno-deficiency virus 1)	Lentivirus	H9 cell line infected with HIV-1	−
HIV-2[a] (human immuno-deficiency virus 2)	Lentivirus	H9 cell line infected with HIV-2	−

[a] Presence of the replication defective viruses RSV and MSV in the respective cell lines is supported by rescue and syncytia formation when co-infected with replication competent retroviruses. Proof that MMTV, MMPV, BLV, HTLV-1,2, and HIV-1,2 were infecting the respective cell lines at the time of testing primer cross-reactivity was provided by a positive reaction of the cell lines with antibodies to the respective virus.

Because of the association between BLV and lymphoma in cattle (3), it was decided to test the alpaca for this virus. Although previous cases of lymphoma in NWC had been screened for retroviruses and none were detected (1,4), the ultrastructural methods used were potentially less sensitive than other tests routinely used in cattle. Methods of detecting BLV infection in cattle include PCR testing and serology (3). Serology was initially used to screen an antemortem blood sample taken from the alpaca, because it offers a rapid and inexpensive preliminary diagnosis, albeit tentative because the methods used are not validated in NWC. This is the first report of antibodies to BLV in NWC; previous surveys did not detect antibodies using the AGIDT in NWC from Argentina and Peru (15,16).

Polymerase chain reaction was selected to confirm the positive serological results. In particular, IS-PCR was selected because the reaction is performed directly on fixed tissue sections, the signal can be localized to a specific cell type and the risk of molecular contamination is eliminated. The primers were chosen to minimize cross-reactivity with other retroviruses yet maximize detection of any BLV variant. Bovine leukemia virus belongs to the retrovirus genus Deltaretrovirus along with human and simian T-cell leukemia viruses (17). Deltaretroviruses contain genomic regions that code for the major structural and enzymatic proteins common to all Retroviridae: *gag, pol,* and *env* (18). In addition, they contain a unique region *tax,* that codes for a viral regulatory protein and is also potentially oncogenic to the host cell. *tax* is not shared by any other genus of retroviruses, including endogenous retroviruses (18,19). The use of *tax* primers in this study eliminated the possibility of cross-reactivity with other retroviruses outside of the Deltaretrovirus genus and this was confirmed by demonstrating non-reactivity in a panel of 8 representatives of other retroviral genera. In addition, the primers did not amplify proviral DNA of other Deltaretroviruses, confirming the specificity of the primers for BLV (Table 1). The presence of BLV proviral DNA in some of the alpaca tissues tested provides the first molecular evidence of infection with BLV in NWC. Although evidence of retroviruses was previously reported in an alpaca by transmission electron microscopy of tissues and reverse transcriptase assay, the virus was not fully characterized (20).

The source of viral infection for this alpaca is unclear. It did not have recent direct contact with other domestic ruminants; however, previous contact could not be ruled out. No other animals on the farm have been serologically tested, but this would be a useful step to determine the prevalence of BLV infection. A causal role for BLV in the pathogenesis of lymphoma could not be established as the virus was not detected within neoplastic cells. Further studies on BLV-positive lymphomatous NWC compared to normal controls would be necessary to establish a causal association.

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Book Review
Compte rendu de livre

Exotic Small Mammal Care and Husbandry

Banks RE, Sharp JM, Doss SD, Vanderford DA. Wiley-Blackwell Publishing, Ames, Iowa, USA. 2010. 192 pp. ISBN 9780-8138-1022-5. $59.99.

This book is written for veterinary technicians or veterinarians desiring in-depth information on the care and prevention of disease in small mammals. In addition, it also provides some basic information on the most common diseases occurring in a variety of caged pets. The information provided would be useful to clinicians and technicians in private practice or for those in a research setting.

The first chapter provides detailed information on intrinsic and extrinsic factors affecting the well-being of caged pets, including topics such as age, gender, genetics, temperature/humidity, ventilation, and illumination. The second chapter focuses on enrichment strategies for common caged pets such as rodents (including mice, rats, hamster, gerbils, and degus), rabbits, guinea pigs, sugar gliders, and hedgehogs. Tables are provided that include normal behaviors that should be considered in the development of an overall strategy of enrichment. The authors also provide specific examples of items that can be used for each species to provide environmental enrichment. The third chapter is devoted to a discussion on common zoonotic diseases of importance in small mammals. Details, such as agent, reservoir and incidence, diagnosis, disease in animals, disease in humans, transmission, and prevention/control are listed for each disease discussed. The remaining chapters of the book each focus on a specific small mammal. Rabbits, ferrets, mice, rats, gerbils, hamsters, guinea pigs, chinchillas, degus, hedgehogs, sugar gliders, and opossums are covered in this book. Basic anatomy and physiology, reproduction, husbandry, nutrition, enrichment, handling/restraint, physical examinations, clinical techniques, preventive health, and common diseases are covered for each of the above-mentioned animals. A basic formulary and clinical pathologic reference values are provided as well.

The main strengths of this book lie in the first three chapters, which provide much information in the husbandry and enrichment of small mammals. Readers desiring more detailed information on common disease conditions and treatments would do better to find another reference source, although at the end of each "animal-specific" chapter, a list of references for further information is provided. There are some color plates and images, though they do little to add to the book. Overall, this book is a useful reference for those veterinarians or technicians working with small mammals.

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